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# Thermophilic Bacteria: a New Cause of Human Disease

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**We studied a group of 31 bacterial isolates from clinical specimens, received by the Centers for Disease Control since 1961, which have been denoted thermophilic for their unusual ability to grow at 50°C. Microbiological characteristics were determined for the group, and an assessment of their clinical significance was made based on retrospective chart review. These bacteria are all gram-negative, nonfermentative, nonsporulating rods, most of which grow better at 42 or 50°C than at 35°C. Some of the bacteria could be implicated as the etiological agents for meningitis, endocarditis, and septicemia. Thermophilic bacteria should be considered potential pathogens when isolated from appropriate clinical specimens.**

The Centers for Disease Control (CDC) Division of Bacterial Diseases receives for identification and classification isolates from reference laboratories throughout the United States. Since 1961, the Special Bacterial Pathogens Laboratory has received 31 bacterial strains which have remained unidentified but which have been noted to have the ability to grow at 50°C. As no known pathogens grow at this temperature, we questioned whether these "thermophilic" bacteria could cause human disease.

## MATERIALS AND METHODS

**Identification of isolates.** Bacteria submitted as unknowns to the Special Bacterial Pathogens Laboratory are grown at a variety of temperatures to determine thermal preference. The 31 strains described in this report were not classifiable into any known species, but each had been noted to have the unusual ability to grow at 50°C. Most grew better when incubated at 42 or 50°C than at 35°C, hence the group designation thermophiles.

**Bacteriological methods.** Thermal preference was determined on tryptone-glucose-yeast extract agar (TGYE) slants incubated at 25, 35, 42, and 50°C for 18 to 24 h. Tolerance of incubation at 65°C was tested in heart infusion broth. Colonial morphology was determined by inoculation on plates of heart infusion agar containing 5% defibrinated rabbit blood (HIA) (10). Gram stains were performed by the Hucker modified procedure on isolates from plates which were 36 to 48 h old (9). Flagellar morphology was determined on growth from TGYE cultures stained with Ryu staining solution (5). Motility was determined by microscopic examination of heart infusion broth cultures or by demonstration of spreading in modified Difco motility medium incubated at 35°C. Spore formation was determined by staining growth from HIA slants, urea agar, esculin agar, and motility medium incubated at 35 or 42°C. Biochemical tests were performed at 35°C. Heat stress was performed by incubation at 80°C for 10 min. Plates were grown in a candle-jar atmosphere, and slants and broths were incubated in air. Medium preparation and biochemical tests were performed as previously described by the Special Bacterial Pathogens Laboratory (4, 10). Antibiotic susceptibility was determined by broth microdilution in cation-supplemented Mueller-Hinton broth incubated at 35°C for 48 h. Susceptibility was defined as the MIC, interpreted by standard guidelines (11).

Cellular fatty acid and isoprenoid quinone determinations were performed on cells harvested from HIA plates. Cellular lipids were saponified and methylated, and the resulting fatty acid methyl esters were analyzed by gas liquid chromatography (GLC) as previously reported (7). Isoprenoid quinones were extracted from saponified cells and analyzed as described previously by reverse-phase thin-layer and high-pressure liquid chromatography (3, 8). Identification of both fatty acids and isoprenoid quinones was confirmed by electron impact and chemical-ionization mass spectrometry (3, 8).

**Retrieval of clinical information.** The 31 isolates had been forwarded by state health department reference laboratories from hospital laboratories requesting identification of unusual organisms. Medical records for the source of each specimen were requested from the originating laboratories. Initial information was obtained by telephone, and when clinical relevance was suggested, copies of pertinent portions of the patient charts were forwarded to the CDC.

## RESULTS

**Sources of specimens.** The thermophilic bacteria were submitted by 17 state laboratories from all regions of the United States and by one laboratory in Belgium. The 31 isolates were received over 22 years without any trends in yearly incidence. A total of 18 isolates were from blood cultures, 3 were from cerebrospinal fluid (CSF), and 10 were from other sites including urine, nasopharynx, pilonidal abscess, wound drainage, and liver biopsy specimens.

Clinical correlation was not possible for 16 of the 31 cases. Five isolates could not be traced to their submitting hospitals, four others could not be matched with patient names, and six patient charts could not be located. The case from Belgium was not investigated.

Clinical information, however, was obtained on 15 cases. Six of the isolates were from normally sterile body sites in

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TABLE 1. Selected characteristics of 31 thermophile isolates studied at the CDC

| Test[a] | No. positive (%) |
|---|---|
| Glucose fermentation | 0 |
| Acid production on O-F media | |
| O-F glucose | 21 (68)[b] |
| O-F maltose | 23 (74)[b] |
| O-F lactose | 0 |
| O-F xylose | 0 |
| O-F sucrose | 0 |
| O-F mannitol | 0 |
| Acid production on TSI slant | 0 |
| Acid production in TSI butt | 0 |
| Hydrogen sulfide production in TSI butt | 0 |
| Growth on MacConkey agar | 0[c] |
| Oxidase | 31 (100) |
| Nitrate reduction | 20 (65) |
| Nitrite reduction | 17 (55) |
| Gas from nitrate | 0 |
| Indole | 0 |
| Flagella (monotrichous or two polar) | 21 (68) |
| Spore formation | 0 |
| Yellow pigment production | 4 (13) |
| Growth on TGYE slants at | |
| 50°C | 31 (100) |
| 42°C | 31 (100) |
| 35°C | 28 (90)[d] |
| 25°C | 0 |

[a] O-F, Oxidation-fermentation; TSI, triple sugar iron.
[b] Does not include four delayed positives at 3 to 7 days.
[c] Does not include three delayed positives at 3 to 7 days.
[d] All 31 isolates could grow at 35°C on HIA plates.

patients whose clinical illnesses suggested a bacterial disease and from whom other bacterial species were not recovered. For the other nine cases, evidence for disease due to thermophilic bacteria was ambiguous or absent.

**Microbiological characteristics.** All of the strains of the thermophiles grew at 50°C on TGYE slants. None of the strains grew at 25°C in 18 to 24 h, and none grew at 65°C when cultured in heart infusion broth for 7 days. No spore formation could be detected, and none of the cultures survived heat at 80°C for 10 min. Of the 31 isolates, 21 were motile. Colonies of the thermophiles were circular, usually 0.5 mm or less in diameter after 48 to 72 h at 35°C, and appeared smooth, convex, semi-translucent, and slightly glossy. Four strains on TGYE produced a distinct yellow pigmentation that was absent from colonies of the other thermophiles; one produced this pigment at 35 and at 42°C, and three produced it only at 42°C. Biochemical reactions and other culture results are listed in Table 1.

TABLE 3. Number of thermophile strains susceptible to antimicrobial agents tested[a]

| Antibiotic | Susceptible | Moderately susceptible | Resistant |
|---|---|---|---|
| Ampicillin | 30 | 0 | 0 |
| Carbenicillin | 30 | 0 | 0 |
| Ticarcillin | 30 | 0 | 0 |
| Piperacillin | 30 | 0 | 0 |
| Mezlocillin | 30 | 0 | 0 |
| Azlocillin | 30 | 0 | 0 |
| Cephalothin | 30 | 0 | 0 |
| Cefamandole | 30 | 0 | 0 |
| Cefoxitin | 30 | 0 | 0 |
| Cefotaxime | 30 | 0 | 0 |
| Cefoperazone | 30 | 0 | 0 |
| Moxalactam | 30 | 0 | 0 |
| Chloramphenicol | 30 | 0 | 0 |
| Tetracycline | 17 | 12 | 1 |
| Erythromycin | 13 | 15 | 2 |
| Trimethoprim/ sulfamethoxazole | 21 | 5 | 4 |
| Gentamicin | 28 | 2 | 0 |
| Tobramycin | 29 | 0 | 1 |
| Netilmicin | 29 | 0 | 1 |

[a] Antimicrobial agent testing was performed on 30 of the 31 strains. Susceptible, moderately susceptible, and resistant were determined by MICs interpreted by National Committee for Clinical Laboratory Standards guidelines (11).

The thermophiles are gram-negative rods of several different morphologies. Twenty-two strains were medium-length, slender bacilli of similar size and shape. All of the four pigmented strains were of this morphology. Five of the remaining nine strains were short, slightly pleomorphic coccobacilli, and four were long, thin rods. Based on their appearance, we have classified the thermophiles into four phenotypes. We designated the nonpigmented, slender strains as type I, the coccobacillary isolates as type II, the thin rods as type III, and the pigmented strains as type IV (Table 2).

**Antimicrobial susceptibilities.** The thermophiles were uniformly susceptible to penicillin and cephalosporin derivatives and, with few exceptions, to the aminoglycosides (Table 3).

**Cellular fatty acid profiles.** The GLC data show two distinct fatty acid groups, one characterized by major amounts of saturated and mono-unsaturated straight-chain acids and one containing major amounts of branched-chain fatty acids. Fatty acid profiles of 15 of the 18 type I isolates were of the first GLC group, which we have designated GLC group A. This pattern also was shared by a single type II strain. The other GLC pattern, which we have called GLC group B, was found in all of the remaining thermophile

TABLE 2. Morphological classification of 31 thermophile isolates studied at the CDC

| Type | No. of isolates | Cellular morphology | Approx dimensions (μm) | Colony pigmentation | Major GLC[a] profile (no. of strains) |
|---|---|---|---|---|---|
| I | 18 | Slender | 0.5 by 1.5 | Nonpigmented | A (15)[b] |
| II | 5 | Short, slightly pleomorphic | 0.5 by 0.5 to 1.5 | Nonpigmented | B (4)[c] |
| III | 4 | Thin | 0.3 by 3 | Nonpigmented | B (4) |
| IV | 4 | Slender | 0.5 by 1.5 | Yellow | B (4) |

[a] See the text for a description of profiles.
[b] Profile B was found in three strains.
[c] Profile A was found in one strain.

TABLE 4. Relative fatty acid composition of 31 thermophile strains studied at the CDC[a]

| Morphological Type | i-11:0 | 3-OH 10:0 | 12:0 | 3-OH i-11:0 | i-15:0 | i-16:0 | 16:1$^{\Delta 9}$ | 16:0 | i-17:1 | i-17:0 | CYC 17:0 | C-V 18:1 | CYC 19:0 | Other fatty acid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GLC group A | | | | | | | | | | | | | | |
| I | – | 7 | 4 | – | – | T | 19 | 25 | – | – | 6 | 20 | T | 19 |
| I | – | 6 | 2 | – | T | T | 8 | 31 | – | – | 7 | 24 | 4 | 18 |
| I | – | 5 | 2 | – | – | T | 10 | 28 | – | – | 6 | 30 | 2 | 17 |
| I | – | 5 | 2 | – | T | T | 16 | 28 | – | – | 6 | 24 | T | 19 |
| I | – | 3 | 2 | – | T | T | 13 | 32 | – | – | 7 | 34 | T | 9 |
| I | – | 5 | 2 | – | T | T | 8 | 29 | – | – | 7 | 26 | 2 | 21 |
| I | – | 6 | 2 | – | – | – | 19 | 31 | – | – | 5 | 26 | T | 11 |
| I | – | 5 | 2 | – | T | T | 9 | 29 | – | – | 8 | 27 | 3 | 17 |
| I | – | 5 | 2 | – | – | T | 14 | 30 | – | – | 3 | 27 | T | 19 |
| I | – | 4 | 2 | – | T | T | 10 | 26 | – | – | 7 | 29 | 3 | 19 |
| I | – | 5 | 2 | – | – | T | 12 | 30 | – | – | 9 | 30 | 2 | 10 |
| I | – | 4 | 2 | – | T | T | 8 | 29 | – | – | 8 | 27 | 3 | 19 |
| I | – | 6 | 2 | – | T | T | 14 | 31 | – | – | 8 | 17 | T | 22 |
| I | – | 5 | 2 | – | T | T | 20 | 26 | – | – | 2 | 38 | T | 7 |
| I | – | 3 | 2 | – | T | T | 8 | 37 | – | – | 24 | 21 | 3 | 2 |
| II | – | – | – | – | – | – | 37 | 39 | – | – | 2 | 15 | T | 7 |
| GLC group B | | | | | | | | | | | | | | |
| I | 5 | – | – | 8 | 51 | 3 | 2 | 2 | 15 | 11 | – | T | – | 3 |
| I | 4 | – | – | 6 | 21 | 24 | T | T | 24 | 15 | – | T | – | 6 |
| I | 3 | 2 | – | 4 | 19 | 2 | 12 | 13 | 14 | 16 | T | 13 | – | 2 |
| II | 3 | – | – | 7 | 43 | 8 | T | 2 | 19 | 10 | – | T | – | 8 |
| II | 6 | – | – | 8 | 52 | 6 | T | T | 13 | 4 | – | T | – | 11 |
| II | 3 | – | – | 8 | 47 | 2 | 3 | 2 | 19 | 14 | – | T | – | 2 |
| II | 6 | – | – | 8 | 47 | 6 | 2 | 2 | 17 | 9 | – | T | – | 3 |
| III | 5 | – | – | 8 | 51 | 5 | T | 2 | 16 | 11 | – | T | – | 2 |
| III | 2 | – | – | 7 | 49 | 6 | 2 | 3 | 15 | 9 | – | T | – | 7 |
| III | 2 | – | – | 7 | 50 | 4 | T | 2 | 17 | 10 | – | T | – | 8 |
| III | 2 | – | – | 7 | 48 | 4 | T | 2 | 17 | 11 | – | T | – | 9 |
| IV | 3 | – | – | 5 | 21 | 4 | T | 5 | 24 | 33 | – | T | – | 5 |
| IV | 4 | – | – | 5 | 28 | 3 | 2 | 3 | 27 | 22 | – | T | – | 6 |
| IV | 3 | – | – | 6 | 32 | 2 | 2 | 5 | 23 | 24 | – | T | – | 3 |
| IV | 4 | – | – | 6 | 17 | 6 | 2 | 6 | 27 | 30 | – | T | – | 2 |

[a] Composition was determined as percentage of total fatty acids. T, Less than 2%; –, not detected. In the table headings the number to the left of the colon refers to the number of carbon atoms, and the number to the right of the colon refers to the number of double bonds. OH, Hydroxy; i, iso acid; Δ9, double bond in 9 position from carboxyl end; CYC, cyclopropane; C-V, *cis*-vaccenic.

strains and is unique from other gram-negative organisms we have studied. In general, strains of each GLC group were homogeneous in cellular fatty acid composition, with a distinct difference between the two groups. Quantitative data for the major fatty acids of the 31 strains are shown in Table 4.

Each of the 31 strains contained ubiquinone Q8 as the major isoprenoid quinone and small-to-trace amounts of Q7 and Q9; no menaquinones were detected (2). The overall isoprenoid quinone content of the 31 isolates is similar to that reported for other gram-negative aerobic rods in which Q8 is the major component.

TABLE 5. Clinical manifestations of six cases of thermophile infection studied at the CDC

| Case | Age (yr) | Sex | Underlying disease | Presentation[a] | Outcome | Source of isolate | Type[b] of Isolate | GLC Group |
|---|---|---|---|---|---|---|---|---|
| 1 | 16 | M | None | URI prodrome, fulminant meningitis | Died after 10 days | CSF | I | A |
| 2 | 4 | F | None | URI prodrome, septic meningitis | Recovered | CSF | I | A |
| 3 | 0.9 | M | Infantile spinal muscular atrophy, ? immunodeficiency | Respiratory distress, 105°F (40.56°C) fever, septic | Died after 2 days | Blood | II | B |
| 4 | 84 | M | Emphysema, organic brain syndrome | Anemia, spiking fever, heart murmur | Recovered | Blood | I | B |
| 5 | 47 | F | Rheumatic heart disease, mitral valve replacement | Cerebral embolism, spiking fever, new murmur | Died after 32 days | Blood | III | B |
| 6 | 64 | F | New-onset stroke | Persistent fever, no localizing findings | Recovered | Blood | I | A |

[a] URI, Upper respiratory infection.
[b] See the text for a description of the thermophile morphological type.

**Clinical manifestations of thermophilic infection.** An illness most probably associated with thermophiles was documented in six cases (Table 5).

Two patients with septic meningitis had thermophiles isolated from their CSF without other bacteria being present; blood culture in one of these cases was positive for the same organism. Both patients had experienced a prodromal illness of 1 to 2 weeks, marked in one case by conjunctivitis and headache and in the other by cough and a "cold." Both occurred in previously healthy children. One recovered after treatment with ampicillin and chloramphenicol, and one died despite intensive antimicrobial therapy and aggressive supportive care.

Respiratory distress, high fever, and sepsis occurred in an infant with infantile spinal muscular atrophy who died within 48 h of admission. Blood cultures were positive for a thermophile on 2 consecutive days without the recovery of other bacteria. Postmortem examination revealed unexpected evidence of giant-cell pneumonia and diminished thymic tissue, suggesting that this infant may have had an immune deficiency that predisposed to bacterial infection.

Two cases of endocarditis occurred in compromised hosts. One was in an elderly nursing-home resident with chronic organic brain syndrome, emphysema, and periodontal pyorrhea. The patient presented with anemia, spiking fever, and a prominent heart murmur of uncertain duration. A thermophile was isolated from two blood cultures taken 1 month apart, before antimicrobial treatment. The patient recovered after 4 weeks of therapy with chloramphenicol. The other patient had rheumatic heart disease with a Starr-Edwards mitral valve prosthesis and atrial fibrillation and had had multiple thromboembolic strokes. Admission was precipitated by recurrence of cerebral embolism, and fever was noted on presentation. Blood culture on one occasion recovered thermophilic bacteria. Despite treatment with ampicillin or penicillin and streptomycin, fever continued and anemia developed. The patient died after 1 month, and necropsy confirmed the presence of verrucous bacterial endocarditis.

One patient had been admitted for acute stroke associated with arteriographically confirmed cerebrovascular disease. She developed a fever during which a thermophile was isolated twice in blood cultures 2 h apart. Her fever persisted for 3 days and then remitted without antimicrobial therapy.

Of the nine isolates of uncertain clinical significance, four were from single blood cultures of patients with fever but no other evidence of infection. These four patients had hemarthrosis, metastatic carcinoma, recent tooth extraction, or coliform pyelonephritis, and each recovered. Another three blood-culture isolates were from afebrile patients who had leukemia, recent pneumonia, and unexplained weight loss; all three patients survived. One isolate was from CSF of a patient with meningismus but no CSF pleocytosis. The remaining isolate was from a liver biopsy obtained because of a clinical impression of infection in a febrile alcoholic; the fever persisted despite antimicrobial therapy, and the patient eventually died, but there was no evidence of bacterial disease noted on necropsy.

## DISCUSSION

The thermophiles are unclassified bacteria that are slow growers even at optimum temperatures, and colonies grown at 35°C on semi-solid media are punctate to 0.5 mm at most by 48 to 72 h. These characteristics can obscure the pathogenic significance of an organism isolated by a clinical laboratory. In at least six cases, however, infection with thermophiles resulted in disease.

In two of these instances the organism responsible was apparently virulent, causing meningitis in previously healthy children. The illness in each was preceded by prodromal upper respiratory symptoms, which may represent a predisposing viral infection or could have been early bacterial symptoms.

In the other four cases the infections occurred in compromised hosts. The infant with septicemia had a progressive paralysis and may have been immunodeficient. Self-limited fever occurred in a patient with a recent stroke. One case of endocarditis occurred in a patient with an intracardiac device, and the other was in an elderly patient who was severely demented.

In all of the cases the mode of acquisition of infection with thermophiles is unknown. Periodontal pyorrhea was present in one case of endocarditis, but the relevance of this observation is unknown.

In all six cases, the isolates were susceptible in vitro to the antimicrobial agents administered, and the three patients who died appeared moribund on admission. In general, the thermophiles are susceptible to most antimicrobial agents in common use.

Bacteria capable of growth at 250°C have been recently isolated from deep-sea hydrothermal systems (1), but among human pathogens, the highest temperature tolerance is recorded for the various species of actinomycetes, which can grow at temperatures as high as 65°C (6). These bacteria, which survive on silage or compost, can cause hypersensitivity pneumonitis if inhaled but do not grow in the human host.

*Campylobacter* species have the highest reported temperature for growth of bacteria isolated from clinical material. They have been known to grow at temperatures of up to 45°C (12). The thermophilic bacteria reported here, most of which grow better at temperatures greater than 35°C, are novel for their ability to grow at 50°C.

Although they were grouped together by a somewhat arbitrary criterion, the thermophilic bacteria have many features in common. The thermophiles are asporogenous gram-negative rods and coccobacilli that are polar monotrichous (or two polar) or are nonmotile. Biochemically, they are oxidase positive and MacConkey negative and do not ferment glucose. In cellular fatty acid and isoprenoid-quinone composition they resemble other glucose-nonfermenting bacteria, such as *Pseudomonas* species.

The 31 strains do not seem to be identical since they vary in appearance. We have recognized four types on the basis of cell and colony morphology. The GLC data indicate two homogeneous groups of cellular fatty acid composition, suggesting a close chemical relationship among strains within each group and a clear difference among the groups. Thus, the 31 isolates may represent as few as two distinct bacterial species.

The thermophilic bacteria have not been previously characterized. They appear to comprise a distinct group of organisms with pathogenic potential which may not be readily recognized in a clinical laboratory. The identification of these organisms to the species level and their relation to other bacteria remain to be determined.

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